Natural diversity in daily rhythms of gene expression contributes to phenotypic variation

Supporting Methods, Discussion, Figures and Tables

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Supporting Methods

Additional information on the phenotyping and genotyping procedures.

Seeds were surface sterilized for 10 minutes in 70 % (vol/vol) ethanol, 5 minutes in 100 % (vol/vol) ethanol and washed three times in sterile water. The seeds were then stratified 3 days at 4°C and plated on MS medium with 2% (m/vol) sucrose. In the case of experiments carried out in monochromatic red light, seeds were left 6 hours in white light to induce germination before being placed in a Percival growth chamber.

F2 individuals from the original mapping population were scored for *GI::LUC* activity and subsequently transferred to soil so that leaf material could be harvested for DNA extraction. Seeds from each individual were collected for analysis of the offspring. DNA was extracted from 100 mg of leaf tissue with the Biosprint robot (Qiagen), following the recommendations of the manufacturer. Genotyping of the F2 and F3 populations was performed by Sequenom inc. using a set of 96 markers that had previously been identified from a pool of 360 polymorphisms.

QTL validation in the F3, F4 and F5 progenies was performed following the same procedure than in the F2. Up to 96 individuals per population were phenotyped, transferred to soil and subsequently genotyped. Genotyping of the segregating regions in F4 and F5 populations was performed with CAPS (Cleaved Amplified Polymorphic Sequences), dCAPS or SSLP (Simple Sequence Length Polymorphism) markers designed based on the polymorphisms identified by Sequenom inc., polymorphism described elsewhere (1), and polymorphism information provided by TAIR (www.arabidopsis.org). Polymorphic regions were PCR amplified, digested with the appropriate restriction enzyme in the case of CAPs and dCAPs, and DNA products were visualized on agarose gels.

Generation of the NILs.

The generation of the NILs was initiated with population F3-1 (Fig. S4). The parent of F3-1 was an F2 plant isolated in the original mapping population and whose genotype is presented in Fig. S4*A*. 63 individuals of population F3-1 were genotyped by Sequenom inc. with the complete 96 marker set. From these data we obtained the parent of population F4-3 that was Col-0 homozygous for most of the genetic background, and that was homozygous Lip-0 or heterozygous at the position of the four *TOG* QTLs (Fig. S4*A*). This extra round of genotyping with the complete 96 marker set also excluded any possible contamination of the population. 192 individuals from population F4-3 were then genotyped with a set of in house markers distributed across the genome and designed based on (1), on the information from Sequenom inc. and on the Arabidopsis database (http://www.arabidopsis.org/). In all the following rounds of genotyping, the genetic background was always checked for Col-0 homozygosity to exclude possible contamination of the populations. The parent of population F5 was isolated from F4-3 as it was almost completely homozygous Col-0 except at the *TOG* loci (Fig. S4*A*). Importantly, all the F3, F4 and F5 families until this point were scored for *GI*::*LUC* expression before genotyping, so that the allelic effect of the *TOG*s was confirmed before introgression of the QTLs. Finally, the parent of the NILs was obtained from the F5 population as it was completely homozygous Col-0 except at the *TOG*s that were Lip-0 homozygous. This individual was backcrossed to Col-0 and a recombination event reduced the size of the *TOG*1 introgression. At this stage all the *TOG*s were heterozygous and individual introgressions of the QTLs could then be obtained in various progenies that we called NILs. We further isolated individuals with combinations of the QTLs in the Col-0 background.

Supporting Discussion

GI peak time is influenced by light signaling and varies at least partly independently of circadian rhythms in Arabidopsis accessions.

In SDs the peak of *GI* expression occurs close to dusk, but in LDs it occurs prior to dusk when plants are still exposed to light (Fig. 1*A* and *C*). We tested whether the peak time of *GI* in SDs was influenced by the onset of darkness at a time when *GI* expression is still rising. We found that in Col-0 plants entrained in SDs of 8 h, an extension of the light period until ZT 16 h delayed the peak of *GI*::*LUC* expression by one hour (Fig. S2*A*). The onset of darkness in SDs therefore causes an abrupt decrease in the transcription of *GI* which results in an apparent peak of transcription close to the time of dusk. This could explain why natural variation of *GI*::*LUC* peak time is limited under SDs, and suggests that a direct effect of light signaling in the evening could contribute to this variation in LDs.

In contrast to peak time, period length measured in DD in the 77 accessions varied to a similar extent after entrainment in all photoperiods tested (Fig. S2*B*). Moreover, period length of plants entrained to 16 h days was equally correlated to that of plants entrained to 14 h or 8 h days (Fig. S2*C*). These results do not support the existence of a mechanism that creates variability of period length specifically in LD photoperiods, as observed for *GI* peak time.

A direct effect of light signaling in the evening alters the waveform of *GI* transcription during LDs.

PHYB regulates the acute response to light of *GI* transcription in the evening (Fig. 2*A* and *B*), and a single 30 min pulse of white or red light during the subjective evening confirmed that PHYB was required for *GI* to fully respond to light (Fig. S8*A* and *B*). After a shift from white light to red light at ZT 8 h in LDs, loss of PHYB activity modified the waveform and delayed the timing of *GI* expression (Fig. S8*D* and *E*). However, after a shift to darkness at the same time of day, the effect of the *phyB-9* mutation on the *GI* waveform was reduced (Fig. S8*D* and *E*). Therefore, the presence of white or red light in the evening of a LD is required for the *phyB-9* mutation to delay *GI* expression. We also studied how changes in circadian rhythms were implicated in the regulation of *GI* peak time during LD conditions. Consistent with previous reports (2), PHYB loss of function in our conditions did not alter the period of *GI::LUC* oscillations in constant DD (Fig. 2*E*) but did lengthen period in constant red light (Fig. S7*E*). Notably, the difference in peak time under red light LDs between Col-0 and *phyB-9* was more than twice the difference in period length detected in red light LL (Fig. S7*D* and *E*), suggesting that the change in period length does not fully account for the change in peak time even in this condition. In our experiments, the effect of PHYB on *GI* was detected in LDs but not in SDs and not in the first day in DD immediately following the LD cycles (Fig. 2*E* and Fig. S7*B* and *C*). Moreover, a second circadian marker, *CCR2::LUC*, which is expressed at a similar time of day to *GI::LUC* was not affected by the *phyB-9* mutation in LDs (Fig. 2*E*). Taken together, the results show that a direct effect of light influences the timing and waveform of *GI* expression in LDs at least partly independently of circadian rhythms.

Alterations of PHYB activity also led to lower absolute expression levels of *GI* in the evening. The *TOG1* Lip-0 allele and the *phyB-9* mutation significantly reduced maximum *GI*::*LUC* expression, generally supporting that PHYB activity promotes *GI* expression in the evening (Fig. S11*B* and *C*). This was confirmed in qRT PCR experiments as evening expression levels of the endogenous *GI* gene was reduced in

phyB-9 and increased in a PHYB overexpressor (Fig. S9*A*). The *phyB-9* mutation also altered *GI* mRNA levels 1 h and 1.5 h after a light pulse as described in Fig. S8*A*, and after a light shift at ZT 8 h as described in Fig. S8*C* (Fig. S9*B*-*D*). *LUC* mRNA behaved similarly to *GI* mRNA which, in addition to the results of the luciferase experiments, confirmed the response to genotype and treatment of the *GI* promoter with two different transcripts (Fig. S9*C*).

Analysis of *GI* expression, circadian rhythms, *PIF4* expression and growth in the NILs

By introgressing combinations of the *TOG* QTLs in the Col-0 genetic background we intended to create a set of individuals that had a similar genetic background but that displayed a range of *GI* peak times and expression levels (Fig. S12*A*). *GI*::*LUC* expression was assayed in four photoperiods and statistical analyses confirmed a significant contribution of the genotypic variation to *GI* peak time and expression levels during LDs (Table S7). Similarly than in the *phyB-9* mutant, *GI* peak time significantly and negatively correlated with *GI*::*LUC* maximum expression in LDs of 16 h but not in SDs (Fig. S12*B*). Importantly, peak time variation in the NILs was not explained by changes in circadian rhythms. First, the contribution of the genotypic variation to variation of period length in DD was weak when significant (Table S7). Second, no significant correlations were detected between *GI* peak time and period length in DD (Fig. S12*B*). Third, period length in constant red light positively correlated with peak time (Pearson test: R=0.695, p=0.015) but as in the *phyB-9* mutant the range of *GI* peak time values was broader than period values (Fig. S12*C*).

GI::*LUC* expression significantly correlated with growth and *PIF4* expression in LDs of 16 h but not in other photoperiods (Fig. 3*D*). Four observations generally supported that the relation between *GI* and *PIF4* expression or growth in the NILs was causal. First, PIF4 activity was required for the long hypocotyl phenotype of *gi-2* (Fig. 3*A*). Second, the analysis of *PIF4* expression in the *gi-2* and *phyB-9 gi-2* mutants demonstrated that GI represses *PIF4* expression in LDs of 16 h (Fig. 3*B*). Third, the correlations of *GI::LUC* expression to hypocotyl length and *PIF4* mRNA in the NILs were negative (Fig. 3*D* and *E*), which is consistent with GI being a repressor of growth and of *PIF4* expression. Fourth, the continuous pattern of the correlations implied that a progressive contribution of reduced *GI* expression levels to increased *PIF4* expression was the scenario that best explained the data (Fig. 3*E*). Changes in PHYB activity in the NILs through allelic variation at *TOG1*, and perhaps at other *TOG*s, could also contribute to the variation of *PIF4* expression by acting synergistically with GI (Fig. 3*C*). The increase in *PIF4* expression, combined with an enhancement of PIF4 stabilization, also due to reduced PHYB activity, could then explain part of the changes in growth (Fig. 4*C*).

Supporting References

1. Berendzen K*, et al.* (2005) A rapid and versatile combined DNA/RNA extraction protocol and its application to the analysis of a novel DNA marker set polymorphic between Arabidopsis thaliana ecotypes Col-0 and Landsberg erecta. *Plant Methods* 1:4.
2. Palagyi A, et al. (2010) Functional analysis of amino-terminal domains of the photoreceptor phytochrome B. *Plant Physiology* 153:1834-1845.
3. Filiault DL, et al. (2008) Amino acid polymorphisms in Arabidopsis phytochrome B cause differential responses to light. *Proc Natl Acad Sci U S A* 105:3157-3162.
4. Johanson U*, et al.* (2000) Molecular analysis of FRIGIDA, a major determinant of natural variation in Arabidopsis flowering time. *Science* 290:344-347.

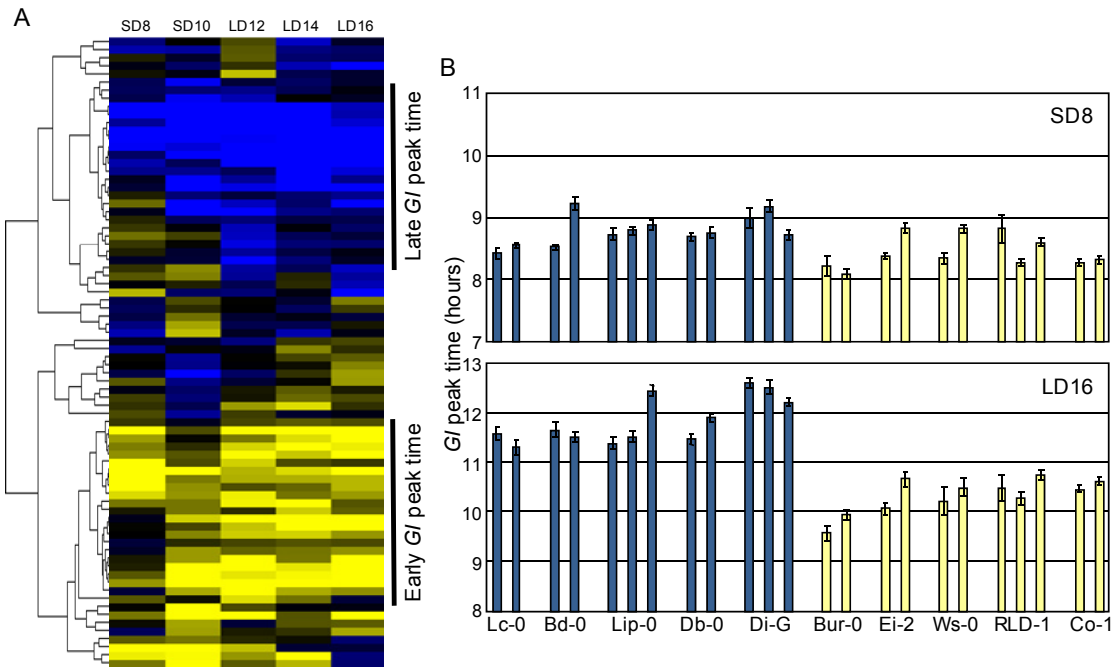

Fig. S1. Natural variation in the timing of *GI* expression in Arabidopsis accessions. (*A*) Hierarchical clustering of *GI* peak times in the accessions. Each row represents an accession and each column represents a photoperiod (SD: short days, LD: long days, number indicates the length of the photoperiod). The data from each photoperiod were mean centered and normalized following the recommended procedure (Cluster version 3). Blue and yellow colors indicate that the *GI* peak time of an accession occurs respectively later or earlier than the average *GI* peak time of all accessions in a photoperiod. Groups of accessions that generally show a late or early *GI* peak time are indicated. (*B*) Example of *GI* peak time data for 10 accessions assayed with multiple transgenic lines. The accessions were selected based on the cluster analysis in *A* and either belong to the group of accessions that generally display a late *GI* peak time (blue bars on the graph), or belong to the group of accessions that generally display an early *GI* peak time (yellow bars on the graph). Results for LDs of 16 h (LD16) and SDs of 8 h (SD8) are shown. These data are a complement to the statistical analysis of Table S2 and illustrate that the insert position did not impede the detection of significant differences in *GI* peak time between accessions. Note that the whole range of variation in LDs 16 h (Fig. 1*A*) is represented in the LD16 panel.

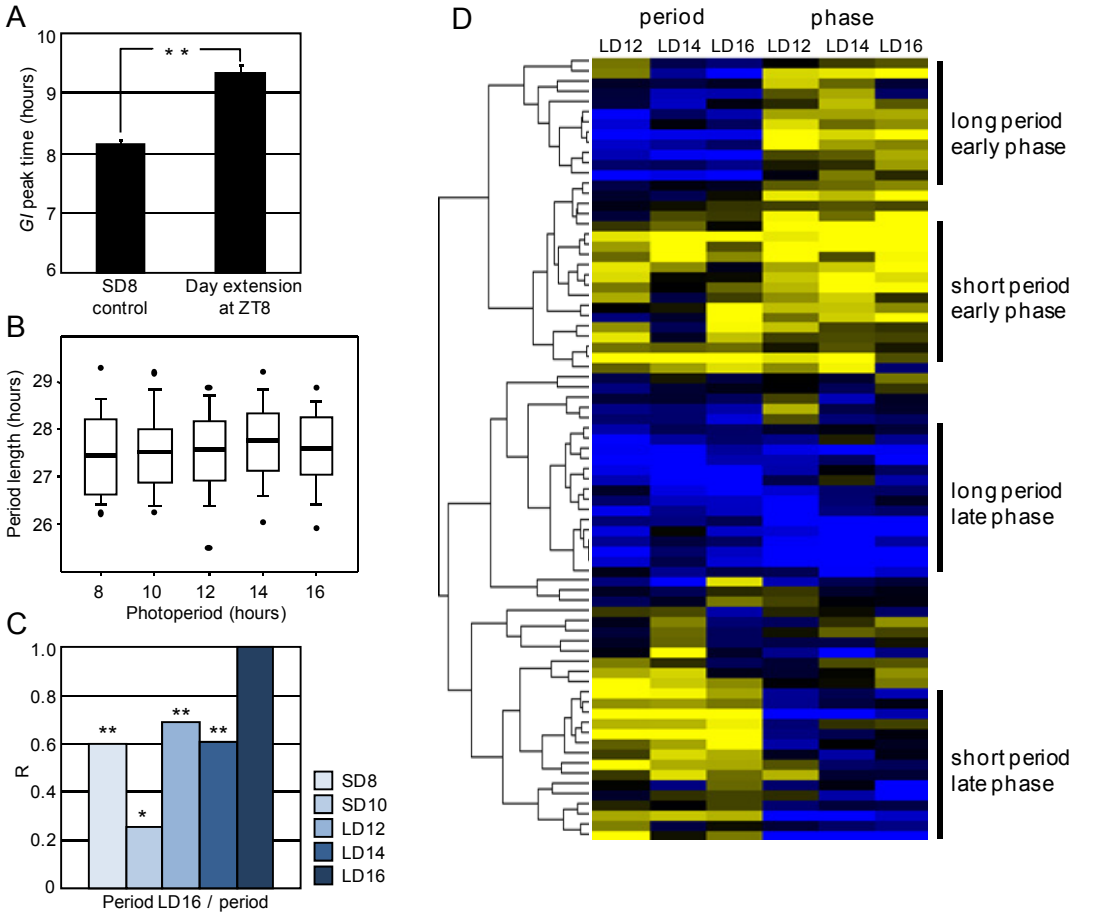

Fig. S2. Light signaling influences the timing of *GI* expression at least partly independently of circadian rhythms. (*A*) *GI* peak time of Col-0 plants entrained in SDs of 8 h and either shifted to darkness or left in white light (day extension) at ZT 8 h on the day of measurement. Error bars represent mean ± s.e.m. **: $p \leq 0.01$ (two tailed Student t-test with $\alpha = 0.05$), $n = 32$. (*B*) Box plots representing the variation of period length in constant darkness (DD) in the 77 accessions after entrainment in 5 photoperiods. Lower and upper limits of the boxes represent 25th and 75th percentile, error bars represent the 10th and 90th percentile and dots represent the 5th and 95th percentile. Horizontal bars represent the mean. (*C*) Correlations between period length in DD after entrainment in LDs of 16 h with period length after entrainment in other photoperiods. The Pearson correlation coefficient (R) indicates the strength of the correlations, 1 and -1 indicating perfect positive and negative correlations respectively. *: $p \leq 0.05$, **: $p \leq 0.01$. (*D*) Hierarchical clustering of *GI* peak time and period length in the accessions. The data from each photoperiod were mean centered and normalized following the recommended procedure (Cluster version 3), and were presented as in Fig. S1*A*. This analysis highlights the existence of two groups of accessions for which period length and phase correlate, and two groups for which they do not.

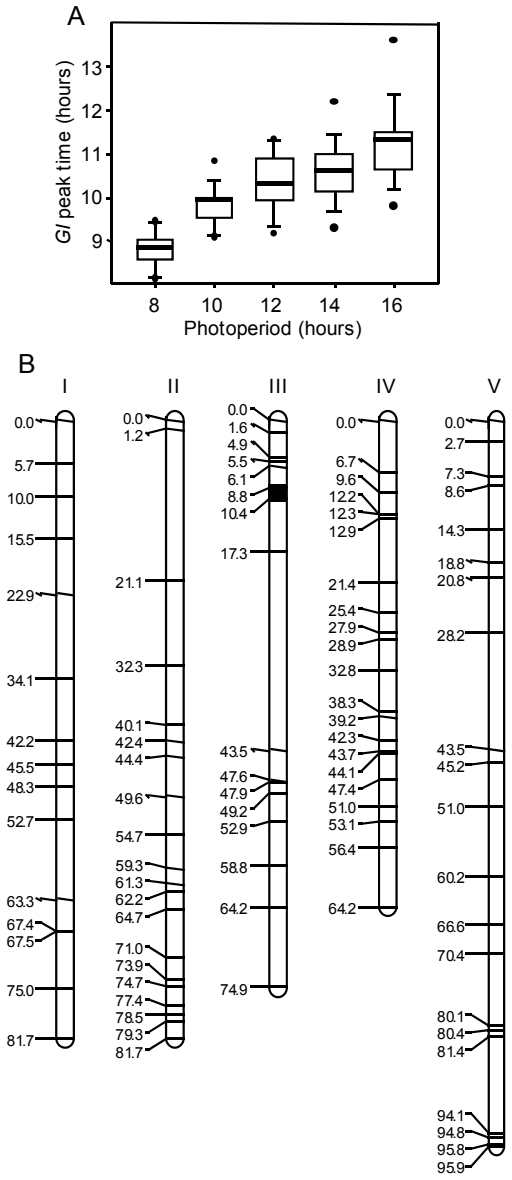

Fig. S3. Phenotypic and genotypic analysis of the Col-0 X Lip-0 *GI::LUC* F2 population. (*A*) Distribution of *GI* peak time measured in Col-0 X Lip-0 F2 progenies grown in five photoperiods. Lower and upper limits of the boxes represent 25th and 75th percentile, error bars represent the 10th and 90th percentile and dots represent the 5th and 95th percentile. Horizontal bars represent the mean. (*B*) Genetic map of the Col-0 X Lip-0 F2 population used for QTL mapping. Genotyping was performed with 96 markers (Sequenom Inc.). Horizontal bars represent markers, and numbers indicate position in cM. Chromosomes are indicated by Roman numerals. The phenotyping of the F2 progenies required selection of the *GI::LUC* transgene, and a strong distortion in the segregation of markers MSQT118 (3679541 bp) and MSQT119 (4141103 bp) revealed the presence of *GI::LUC* on the upper arm of chromosome III (black rectangle). In the original cross the *GI::LUC* transgene was present in the Lip-0 accession, so that all the individuals except one were either heterozygous or homozygous for Lip-0 at the position of MSQT119. No other distortions were detected.

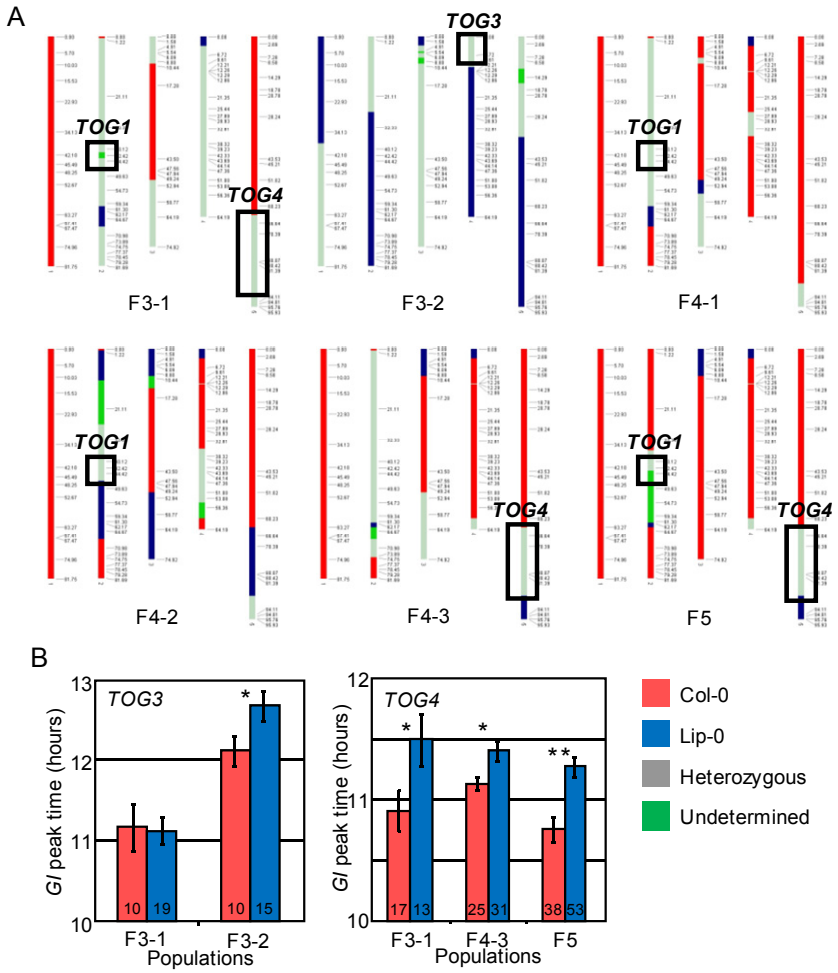

Fig. S4. Detection and confirmation of the *TOG1*, *TOG3* and *TOG4* QTL effects in F3, F4 and F5 populations. (*A*) Genotypes of the parents of the populations used to detect and confirm the QTLs. Boxes indicate the positions of the QTLs. The F3-1 and F3-2 populations were obtained by self-fertilization of F2 plants isolated in the original F2 mapping population. Populations F4-1, F4-2 and F4-3 were obtained after self-fertilization of F3 individuals isolated in F3-1. The F3-1 population was genotyped with the 96 marker set as described for the F2 population in Fig. S3 (Sequenom Inc.). The F5 population was similarly obtained from an F4 plant isolated in F4-3. F4 families were genotyped with in-house markers. (*B*) Markers polymorphic between Col-0 and Lip-0 were designed on chromosomes IV and V to determine the allelic effects of *TOG3* and *TOG4* in the different populations. The effect of *TOG3* was detected in F3-2 but not in F3-1, suggesting that *TOG3* was not segregating in F3-1 and that it was included in the Lip-0 homozygous region at the top of chromosome IV in this population. This region was introgressed in NILs which allowed confirming the effect of *TOG3* (Fig. S5). Because *TOG2* was linked to the *GI::LUC* transgene (Fig. S3), this QTL could not be convincingly confirmed using this approach. (mean ± s.e.m., *n* is indicated on the histograms, *: $p \leq 0.05$, **: $p \leq 0.01$ with a two tailed Student t-test).

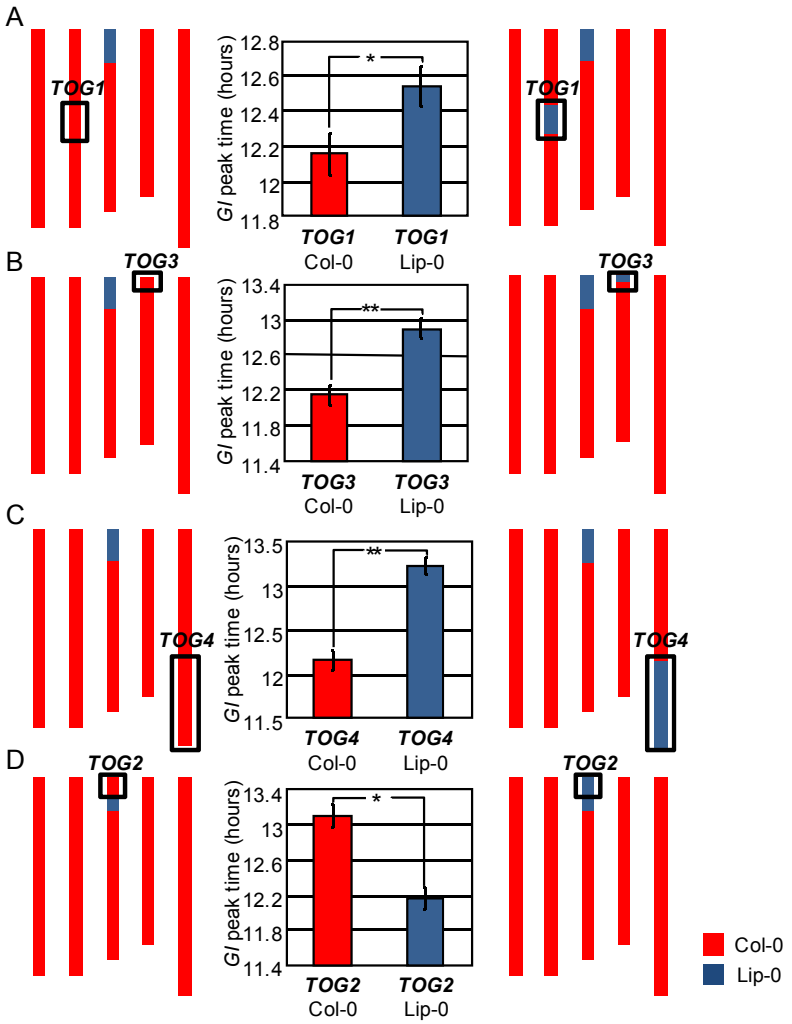

Fig. S5. Confirmation of the effect of (*A*) *TOG1,* (*B*) *TOG3*, (*C*) *TOG4* and (*D*) *TOG2* QTLs in NILs. The peak time of *GI::LUC* expression was determined in the NILs grown in LDs of 16 h. Data were obtained from 5 independent experiments with 12 individuals per genotype per experiment. mean ± s.e.m., p value was determined with a two way ANOVA with genotype and experiment as factors, *: $p \leq 0.05$, **: $p \leq 0.01$. The NILs were generated as described in *SI Material and Methods*, and the sizes of the introgressions are provided in Table S6. For the confirmation of *TOG2*, a recombination event was necessary to combine *TOG2* Col-0 with the *GI::LUC* transgene because in the original cross *GI::LUC* came from the Lip-0 accession and was linked to *TOG2*.

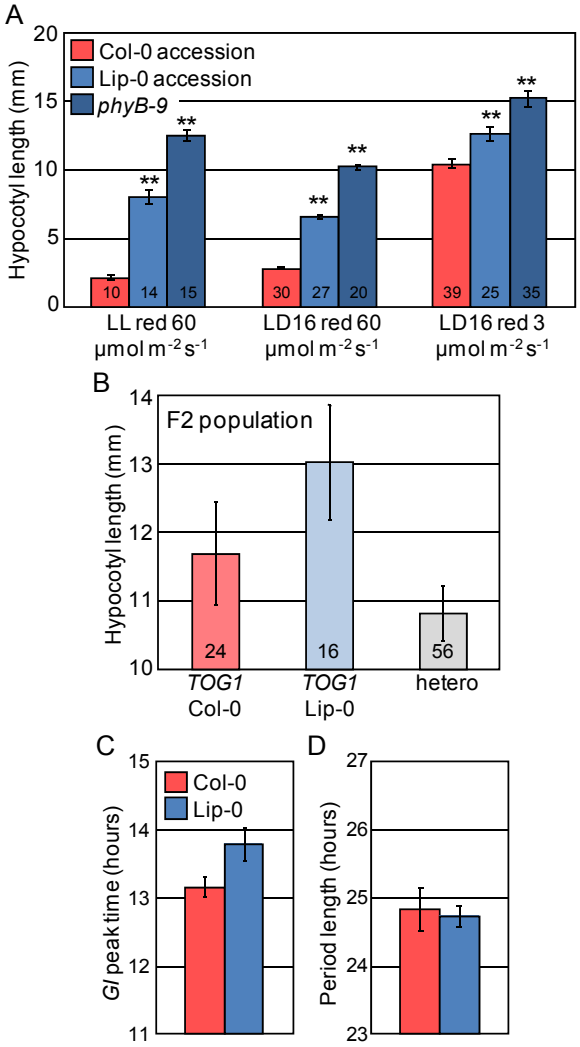

Fig. S6. PHYB activity is altered in the Lip-0 accession and in the F2 population. The Lip-0 genomic sequence of *PHYB* was determined and revealed a 12 nucleotide in frame deletion compared to the Col-0 allele at the 5' end of the coding sequence. This deletion had previously been proposed to reduce PHYB activity (3). (*A*) and (*B*) We confirmed this in our material and experimental conditions. (*A*) Growth phenotype of the Lip-0 accession in constant red light of 60 µmol m^{-2} s^{-1} and in LD 16 h cycles of red light of 3 and 60 µmol m^{-2} s^{-1}. (*B*) Allelic effect of *TOG1* on hypocotyl length in F2 progenies grown LD 16 h cycles of red light of 3 µmol m^{-2} s^{-1}. In (*B*) hypocotyl length of individuals Lip-0 homozygous at *TOG1*, but not of individuals Col-0 homozygous at *TOG1*, were statistically different from the hypocotyls length of heterozygous individuals (two tailed Student t-test). This suggested that *TOG1* Col-0 was the dominant allele. (*C*) Col0 and Lip-0 accessions were entrained in LDs 16 h of red light 60 µmol m^{-2} s^{-1}, and *GI* peak time was determined on day 10 with a CCD camera. (*D*) Plants were then released in constant red light (LL) (60 µmol m^{-2} s^{-1}) and period length was measured. The results of two biological replicates are shown. mean ± s.e.m (n = 4). For (*A*) and (*B*) n is indicated on the bar graphs. mean ± s.e.m., *: $p \leq 0.05$, **: $p \leq 0.01$ with a two tailed Student t-test.

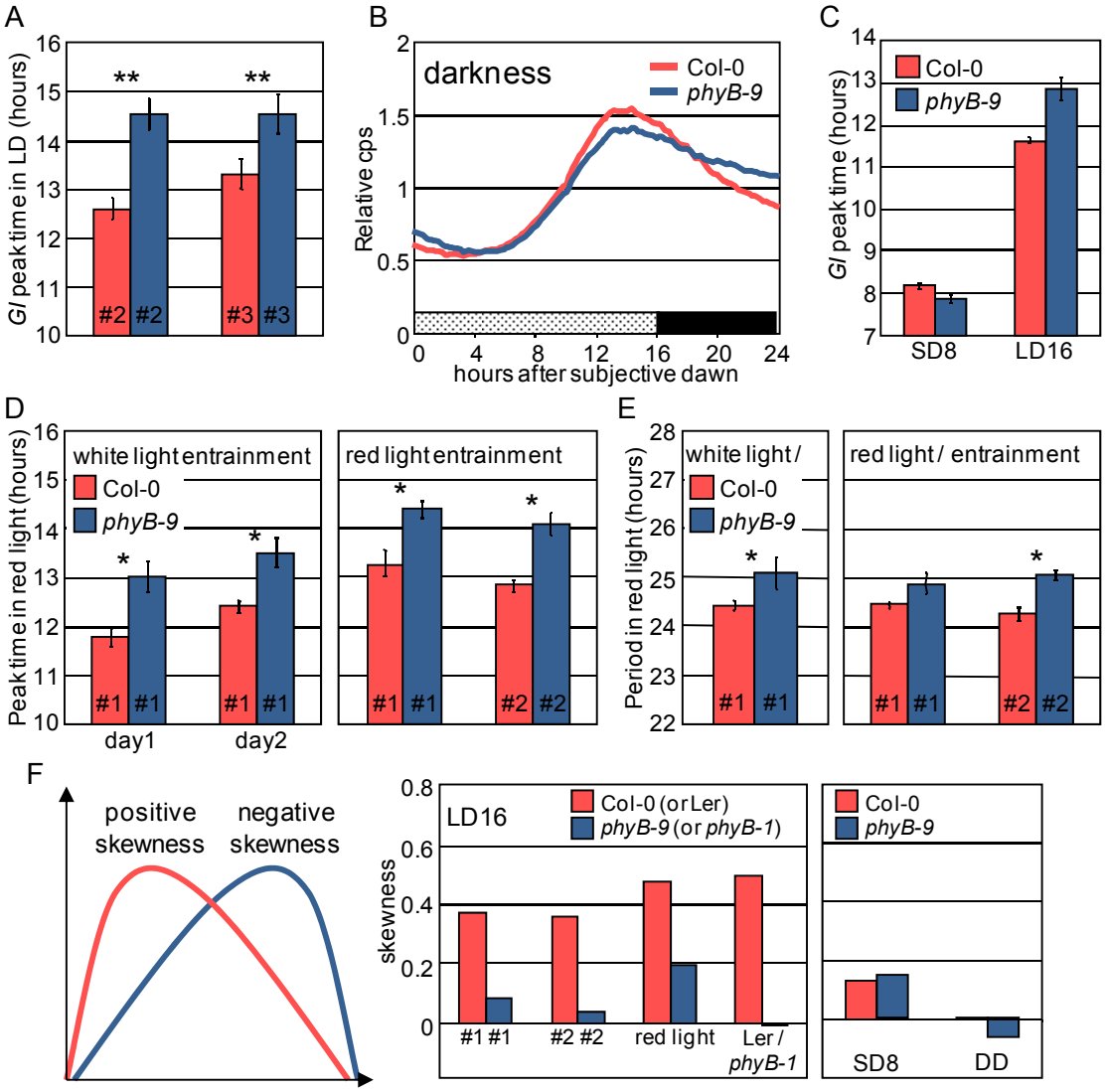

Fig. S7. PHYB activity defines the waveform of *GI* expression in LDs of 16 h. (*A*) Peak time of *GI::LUC* expression measured in independent transgenic lines described elsewhere (35) and not included in Fig. 2. (*B*) Wavefom of *GI::LUC* expression in darkness after entrainment in LDs of 16 h and transfer to DD conditions. (*C*) *GI* peak time measured in Col-0 and *phyB-9* after entrainment in LDs of 16 h and SDs of 8 h (LD16 and SD8) ($n = 32$). (*D*) *GI* peak time measured in red light 60 µmol m^{-2} s^{-1} LDs after entrainment in white light photocycles (results of two consecutive red light LDs are shown) or after entrainment in red light LDs since the first day (results of two transformant lines are shown) ($n = 8$). (*E*) Plants were then released in constant red light (LL) (60 µmol m^{-2} s^{-1}) and period length was determined. (*F*) Skewness of the *GI::LUC* waveforms after entrainment in white light LDs of 16h. The skewness of the curves is also indicated for red light LDs of 16 h, for SDs of 8 h and for darkness as indicated on the figure. mean ± s.e.m., *: $p \leq 0.05$, **: $p \leq 0.01$ with a two tailed Student t-test.

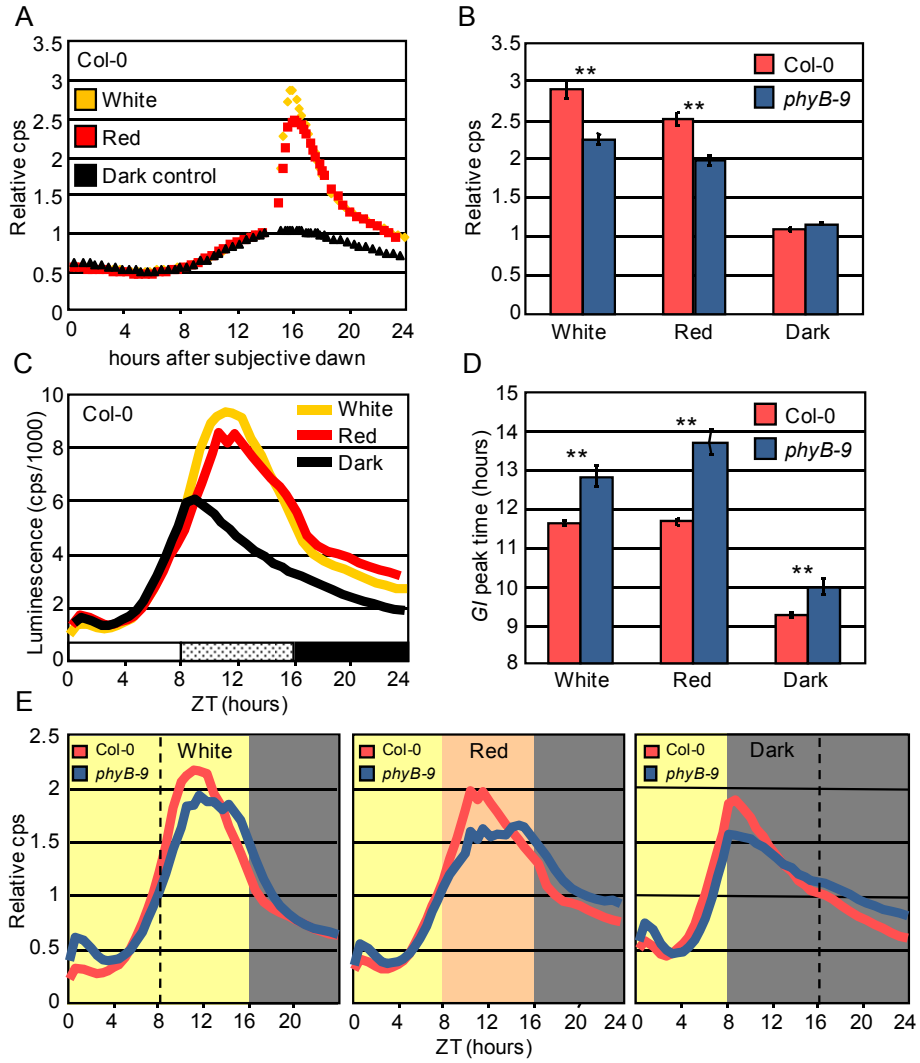

Fig. S8. PHYB activity mediates the acute response to light of *GI* in the evening of a long day. (*A*) Response of Col-0 *GI::LUC* to a 30 minute white or red light pulse applied in the dark 14 hours after subjective dawn. The response was expressed relative to the luminescence measured before the pulse. (*B*) Maximum relative luminescence of Col-0 and *phyB-9* after the light pulse. (*C*) The *GI::LUC* waveform was determined in Col-0 during a LD of 16 h after plants were shifted at ZT 8 h either to 60 µmol m^{-2} s^{-1} red light, either to darkness, or left in white light. Luminescence is expressed in cps = counts per second. (*D*) *GI* peak time measurements in Col-0 and *phyB-9* and (*E*) *GI::LUC* waveform after the shift to different light qualities at ZT 8 h. *n* = 12-24. mean ± s.e.m., *: $p \leq 0.05$, **: $p \leq 0.01$ with a two tailed Student t-test.

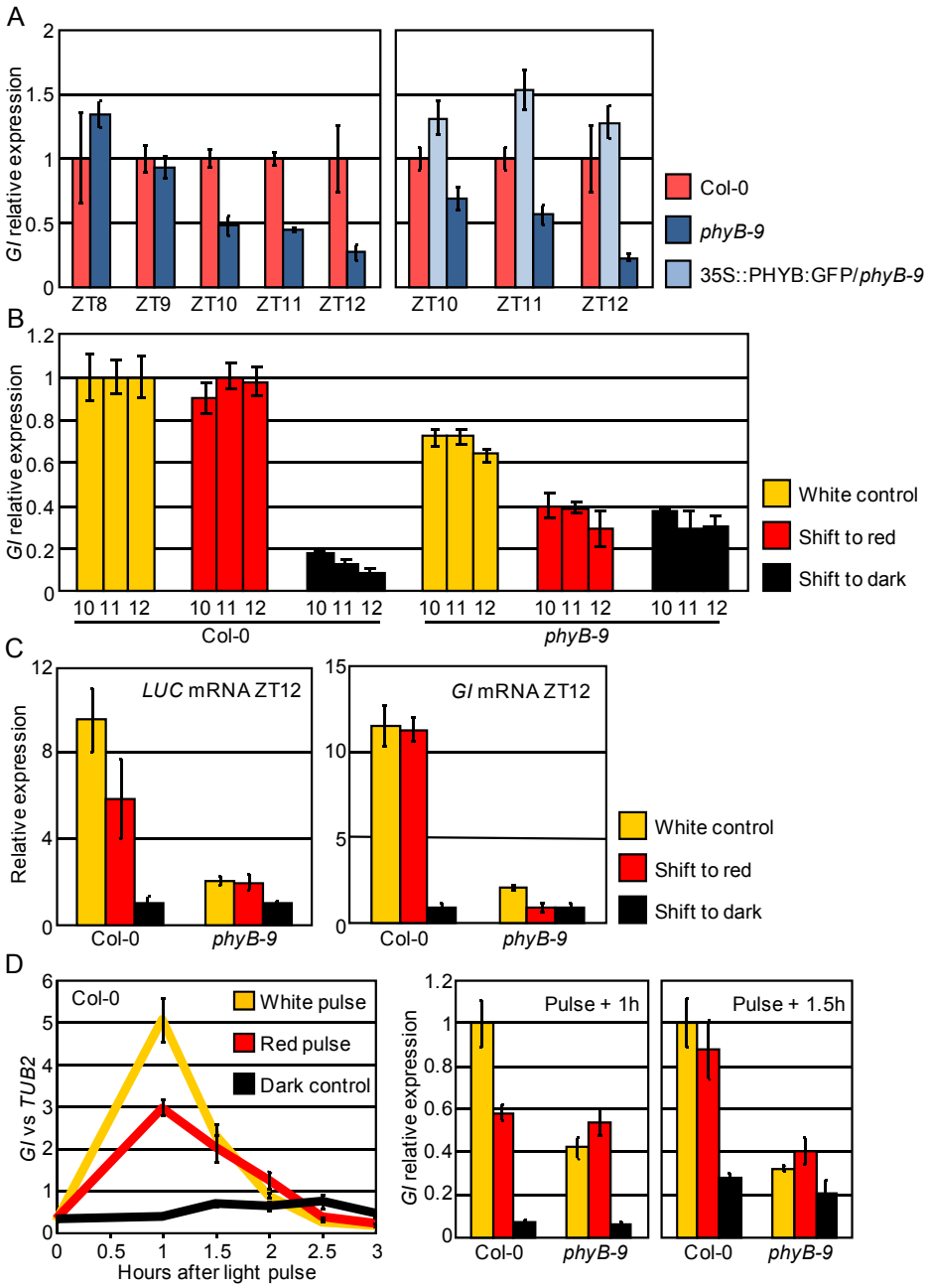

Fig. S9. Reduced PHYB activity alters *GI* expression levels in the evening of an LD of 16 h. (*A*) *GI* mRNA levels determined by qRT PCR in Col-0, *phyB-9* and *35S::PHYB:GFP phyB-9* samples harvested in the evening of a LD of 16 h at the indicated times. *35S::PHYB:GFP* was previously described in (35). (*B*) *GI* mRNA levels determined by qRT-PCR in parallel to the experiment described in Fig. S8*C*. Samples were harvested after the shift to the different light conditions at ZT 10, 11 and 12 h. (*C*) *LUC* mRNA and *GI* mRNA levels determined at ZT 12 h in the same samples than in (*B*) and expressed relatively to the dark control. (*D*) *GI* mRNA levels in samples harvested in parallel to the experiment described in Fig. S8*A*. Results for Col-0 and *phyB-9* were compared 1 and 1.5 hours after the pulse. mean ± s.d. of four technical replicates.

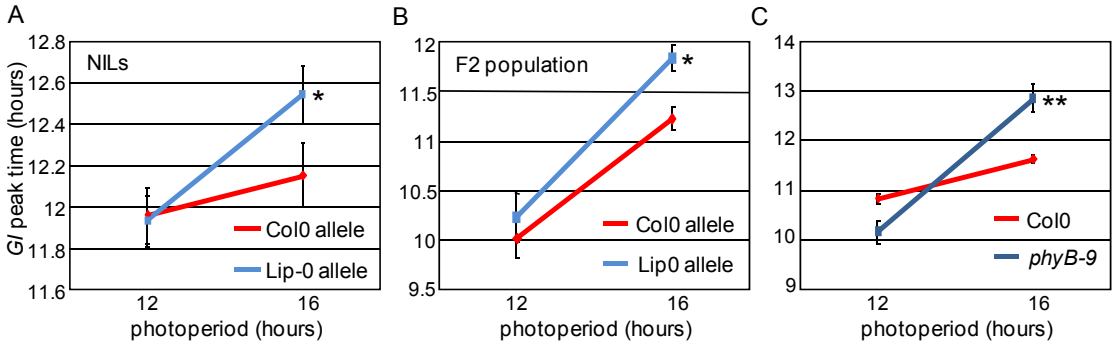

Fig. S10. The *PHYB* Col-0 allele advances *GI* peak time specifically in LDs of 16 h. *GI* peak time of expression was determined in LDs of 12 h and compared to the results in LDs of 16 h for (*A*) the NILs in which the *TOG1* allelic effect had been confirmed (Fig. S5*A*), (*B*) the Col-0 X Lip-0 GI::LUC F2 population, and (*C*) Col-0 and the *phyB-9* mutant. The delay in *GI* peak time due to reduced PHYB activity was not detected in LDs of 12 h. (*A*) Data are the mean of five biological replicates with n = 12 per experiment. (*B*) *GI::LUC* expression was monitored in 80 to 90 F2 individuals and subsequently genotyped for allelic variation at *PHYB*. The average peak time for Col-0 and Lip-0 homozygous plants was determined. (*C*) n = 32. mean ± s.e.m.. * $p \leq 0.05$. p was determined with a two tailed Student t-test (α=0.05).

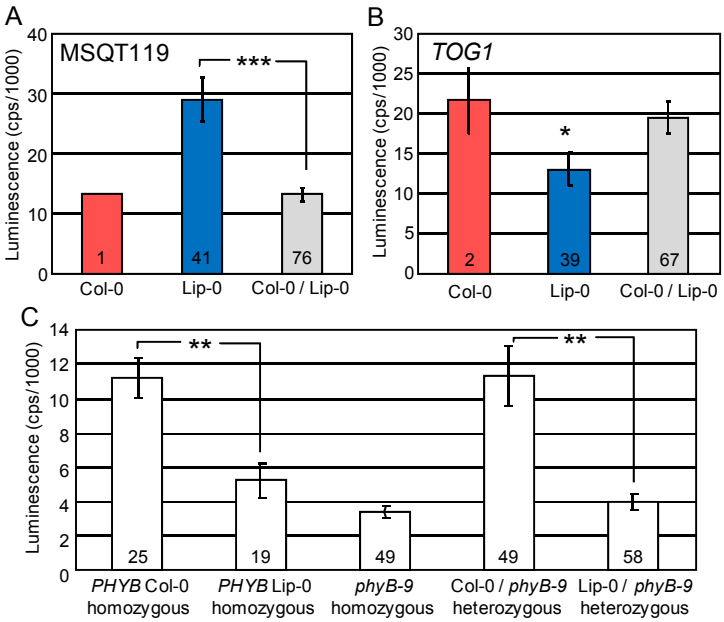

Fig. S11. *PHYB* is the gene underlying *TOG1*. (*A*) Allelic effect of marker MSQT119 on *GI::LUC* expression levels at peak time in the Col-0 X Lip-0 *GI::LUC* F2 population. MSQT119 is linked to *GI::LUC* (Fig. S3), so that the allelic effect of this marker reflects the dosage of the transgene. Individuals that are Lip-0 homozygous at MSQT119 displayed approximately 2 fold higher LUC activity than individuals heterozygous at MSQT119. This result shows that the variation of *GI::LUC* expression level at peak time can be associated to allelic variation at specific loci. *n* is indicated inside the bars of the histogram. (*B*) *GI::LUC* expression in Col-0 X Lip-0 F2 progenies heterozygous, homozygous Col-0, or homozygous Lip-0 at *TOG1*. Individuals Lip-0 homozygous at *TOG1* displayed significantly lower *GI::LUC* expression levels than individuals Col-0 homozygous or heterozygous at this loci. Moreover, *GI::LUC* expression was not significantly different between individuals Col-0 homozygous or heterozygous at *TOG1*, showing that the Col-0 allele was dominant. (*C*) Allelism test performed by crossing the NILs in which the *TOG1* allelic effect had been confirmed (Fig. S5*A*) to the *phyB-9* mutant. The effect of the segregating *PHYB* alleles was determined in families resulting from these crosses and in which the *GI::LUC* transgene located on top of chromosome III had previously been made homozygous. In this experiment, the individuals were first scored for *GI::LUC* activity and subsequently genotyped for the different *PHYB* alleles to determine the allelic combination present in each seedling. *n* is indicated in the bars of the histogram. mean ± s.e.m. *: $p \leq 0.05$, **: $p \leq 0.01$, and ***: $p \leq 0.001$ with a two tailed Student t-test.

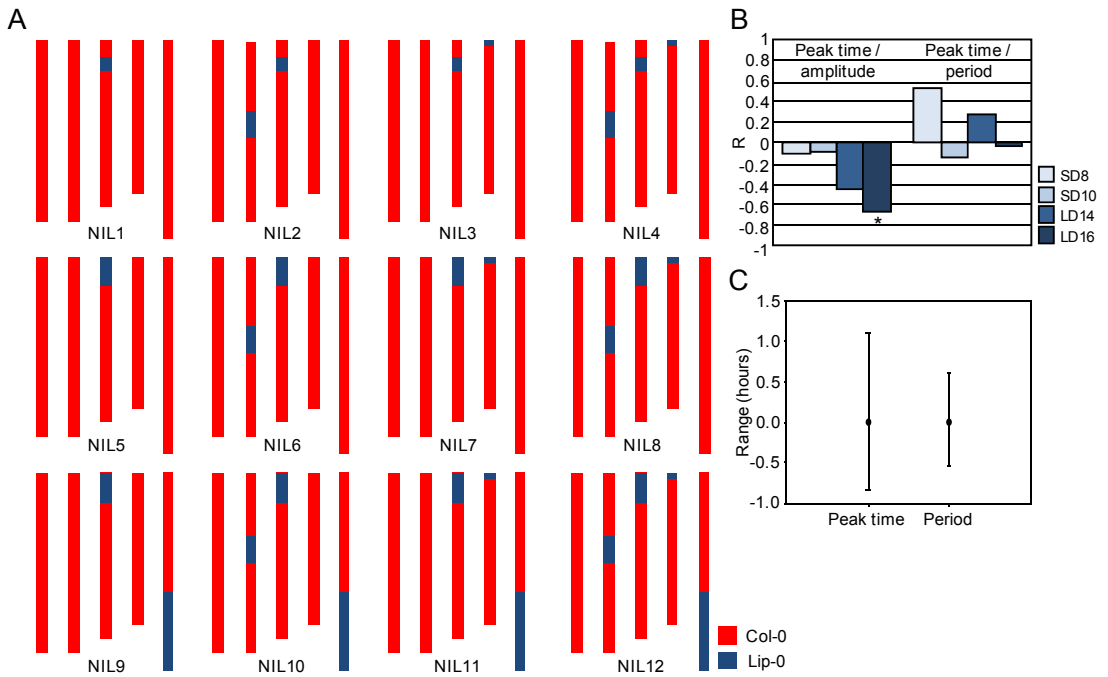

Fig. S12. Natural *TOG* alleles modify the timing and the evening expression level of *GI* expression. (*A*) Schematic representation of the genotypes of the 12 NILs bearing combinations of *TOG1-4* introgressions. (*B*) Correlations between *GI* peak time and *GI* maximum expression levels, and between *GI* peak time and period length in the NILs grown in four photoperiods. Period length was determined in DD after entrainment in the different light regimes. The Pearson correlation coefficient (R) indicates the strength of the correlations, 1 and -1 indicating perfect positive and negative correlations respectively. (*C*) Range of *GI* peak time of expression and period length in the NILs entrained in 60 µmol m^{-2} s^{-1} red light photocycles. Period length was determined in constant red light after entrainment (n = 8). mean ± s.e.m., *: $p \leq 0.05$, **: $p \leq 0.01$ with a two tailed Student t-test.

Table S1. List of Arabidopsis accessions used in this study.

	GI peak time (hours)					*GI* period length (hours)				
	SD8	SD10	LD12	LD14	LD16	SD8	SD10	LD12	LD14	LD16
Aa-0	8.84	9.67	9.97	11.09	11.10	27.71	27.97	27.84	28.00	28.01
Ak-1	8.59	9.84	10.43	10.73	11.20	26.58	27.52	27.38	27.80	27.28
An-2	8.00	9.53	9.55	9.56	9.79	25.23	27.23	26.44	26.04	26.21
Ba-1	8.56	9.73	9.98	10.31	10.78	27.85	28.15	27.67	27.71	27.35
Bay0	8.46	8.86	9.93	10.06	11.29	29.35	29.73	27.85	28.64	28.38
Bch-1	8.57	9.41	10.52	10.79	11.52	27.41	27.40	26.24	26.63	26.84
Bd-0	8.89	10.30	10.89	11.33	11.57	26.96	26.89	26.73	26.85	26.77
Be-2	8.64	9.94	10.14	10.37	10.74	28.24	27.99	26.98	28.14	28.13
Berkeley	8.61	9.38	9.52	10.23	10.21	26.23	26.99	27.84	27.43	27.32
Bla-3	8.76	9.46	10.26	10.61	11.16	28.70	27.70	28.40	28.18	28.06
Br-0	8.45	9.59	9.64	9.76	10.34	27.58	26.49	26.48	27.75	27.54
Bs-1	8.56	9.69	10.58	10.58	11.27	28.43	27.28	28.69	28.99	28.89
Bs-2	8.15	9.53	9.65	10.35	10.84	27.98	27.87	26.84	28.18	26.45
Bs-5	8.71	9.38	9.91	10.24	10.59	26.86	28.20	27.92	27.85	27.75
Bsch-0	8.63	9.36	10.28	10.33	10.74	27.55	27.93	26.94	27.57	27.99
Bsch-2	8.69	9.28	9.42	9.43	9.83	27.95	28.06	26.79	25.94	27.22
Bu-0	9.22	10.42	11.12	11.32	12.35	27.11	27.69	28.14	28.38	27.98
Bu-2	8.79	9.59	10.15	10.81	10.83	27.28	27.58	28.18	28.02	27.70
Bur-0	8.03	9.14	9.72	9.96	10.24	26.44	26.26	26.43	27.13	27.71
C24	8.51	9.10	9.60	9.88	10.04	26.56	26.68	26.94	28.48	28.86
Cen-0	8.60	9.76	10.51	10.74	11.05	26.45	27.04	26.84	27.05	27.05
Chi-0	8.60	9.75	9.93	10.85	11.25	26.57	26.60	26.20	26.97	26.82
Cl-0	8.47	9.55	10.60	10.79	11.15	27.88	29.18	28.17	29.67	26.56
Co-1	8.22	9.44	9.73	10.08	10.49	28.37	27.14	28.79	28.24	28.38
Col-0	8.64	9.67	10.10	10.36	11.33	26.65	26.47	26.46	26.92	26.09
Col-2	8.68	9.86	10.09	10.31	10.92	27.53	27.96	26.99	27.32	27.09
Col-3	8.74	9.59	9.98	10.33	10.79	26.57	25.96	26.43	27.95	26.70
Ct-1	8.64	9.15	9.97	10.58	11.04	27.64	28.86	28.00	27.95	27.05
Da-0	8.65	9.93	10.16	10.52	10.63	26.62	29.08	25.46	26.84	26.95
Da(1)-12	8.46	8.74	9.46	10.33	10.26	26.27	26.69	27.04	26.34	26.96
Db-0	8.73	10.22	10.69	11.33	11.89	27.95	27.47	28.64	27.75	28.50
Di-G	9.04	10.08	11.54	12.09	12.38	28.35	29.43	28.79	28.27	28.48
Di-M	8.60	8.92	9.04	9.93	9.98	28.41	27.24	28.84	28.87	28.65
Dr-0	8.51	9.43	10.50	10.46	11.42	28.48	28.92	29.68	28.53	28.07
Dra-0	10.50	10.62	11.08	11.54	11.50	24.32	26.26	25.28	26.38	25.12
Edi-0	8.76	10.37	10.38	10.42	10.56	27.66	26.81	27.69	27.17	28.11
Ei	8.41	8.95	9.18	9.57	10.19	26.15	26.79	27.29	27.29	27.56
Ei-2	8.65	9.42	9.66	9.98	10.58	27.20	27.00	27.57	27.68	26.26
Eil-0	8.66	9.61	10.34	10.44	11.49	28.68	28.52	28.44	29.52	29.42
El-0	8.64	9.54	9.68	9.65	10.25	26.51	27.36	26.96	27.79	27.12
En-2	8.51	9.92	10.27	10.54	10.57	27.12	27.67	26.71	26.79	27.71
En-D	8.57	9.82	9.78	9.95	10.86	27.13	27.74	26.71	28.10	27.30
En-T	8.85	9.36	10.33	10.77	10.93	26.90	27.10	27.74	27.31	28.01
Est	8.15	9.40	9.96	10.28	10.49	28.65	26.72	28.44	28.92	29.24
Et-0	8.55	9.89	10.10	10.27	10.79	26.67	27.74	27.84	27.29	28.01
Fr-4	8.93	9.31	10.23	11.03	11.05	26.57	27.01	27.25	26.76	26.79
Gr	8.46	9.45	9.57	9.69	10.76	27.35	26.87	25.74	26.43	25.38
Gre-0	8.68	9.86	10.03	11.04	11.83	26.48	27.46	27.77	27.55	27.28
H55	8.70	10.20	10.50	11.30	11.37	27.96	27.44	27.64	24.97	27.78
Hi-0	8.79	9.89	10.47	10.78	11.03	26.66	26.88	25.51	25.91	25.88
Hs-0	8.92	9.96	9.94	10.90	11.27	28.29	27.76	28.09	28.29	28.58
Je54	9.49	10.32	10.78	11.37	11.47	27.70	27.76	28.29	28.07	28.04
Lc-0	8.56	9.66	10.68	10.87	11.29	27.09	27.21	27.76	28.84	28.88
Li-1	8.77	10.13	10.57	10.55	11.09	26.54	27.01	27.09	27.01	26.04
Lip-0	8.81	10.20	11.05	11.93	12.08	28.22	28.65	28.56	28.13	28.56
Lm-2	8.60	9.89	10.29	10.85	11.38	27.33	28.00	26.93	28.09	27.53

Lu-1	8.36	9.86	10.42	10.60	11.96	26.65	26.40	27.54	28.38	27.11
Mt-0	8.62	9.71	9.69	10.77	11.12	27.74	27.24	28.22	28.30	28.37
Nd	8.50	9.45	10.05	10.52	11.29	27.46	27.66	27.27	27.44	28.36
No-0	9.13	10.22	10.73	11.36	11.71	28.74	28.68	28.76	29.19	28.30
Ob-0	8.53	9.95	10.00	10.68	11.05	27.42	28.01	27.73	28.18	27.40
Oy	8.69	10.21	10.80	10.92	11.30	27.15	27.35	28.71	28.44	28.47
Petergof	8.51	9.64	9.62	10.25	11.15	27.51	28.30	27.76	28.10	27.77
PHW	8.87	9.94	10.36	11.28	11.59	26.53	26.36	27.68	28.45	27.93
RLD-1	8.61	9.45	10.04	10.00	10.73	26.40	29.39	27.87	28.69	27.67
Rsch-0	8.77	9.53	10.14	10.69	10.65	26.37	27.63	27.93	27.67	27.88
Rubez-1	8.30	9.39	9.58	10.24	10.57	28.41	28.79	28.61	27.84	28.02
S96	8.92	9.84	10.93	12.07	12.17	28.17	27.45	25.46	27.70	27.03
Sha	8.04	9.07	9.69	10.70	11.15	27.68	28.21	27.28	26.74	27.09
Sn(5)-1	8.79	9.37	10.08	10.42	10.53	29.31	24.21	28.09	28.22	28.23
Sol-0	8.74	9.35	9.39	10.09	10.60	28.60	26.23	28.55	28.50	28.07
Ta	8.48	10.30	10.60	10.84	11.75	30.20	28.44	29.49	29.86	28.39
Tsu-0	8.42	9.51	9.55	10.01	10.19	28.32	27.34	27.78	28.17	27.53
Wil	8.71	9.77	10.84	10.88	11.10	28.34	27.49	28.08	28.68	28.49
Ws-0	8.40	9.66	9.86	9.95	10.22	26.67	26.35	28.15	27.99	25.93
Yo	8.27	9.20	9.79	9.84	11.31	26.78	26.69	27.08	27.03	26.57

Average *GI* peak time during the day and period length in constant darkness is given for every accession in the five photoperiods tested.

Table S2. Statistical analysis of *GI* peak time and period length in the accessions.

***GI* peak time**

LD16	Df	SS	MS	F	% Exp	P
Accession	38	458.24	12.06	28.203	26.9	<1E-15
Accession/Trans	57	366.38	6.43	15.033	21.5	<1E-15
Residuals	2055	878.66	0.43			
LD12	Df	SS	MS	F	% Exp	P
Accession	38	503.26	13.24	31.756	30.6	<1E-15
Accession/Trans	57	293.86	5.16	12.362	17.9	<1E-15
Residuals	2034	848.28	0.42			
SD8	Df	SS	MS	F	% Exp	P
Accession	38	116.79	3.07	12.525	16.8	<1E-15
Accession/Trans	55	103.6	1.88	7.676	14.9	<1E-15
Residuals	1940	476.05	0.25			

Period length

LD16	Df	SS	MS	F	% Exp	P
Accession	37	1449.8	39.2	10.814	17.2	<1E-15
Accession/Trans	49	536.3	10.9	3.0206	6.4	<1E-15
Residuals	1778	6442.4	3.6			
LD12	Df	SS	MS	F	% Exp	P
Accession	37	1762.9	47.6	11.6162	17.4	<1E-15
Accession/Trans	52	766.2	14.7	3.5926	7.6	<1E-15
Residuals	1854	7604.4	4.1			
SD8	Df	SS	MS	F	% Exp	P
Accession	37	1288	34.8	8.4336	13.9	<1E-15
Accession/Trans	50	792.7	15.9	3.8408	8.5	<1E-15
Residuals	1747	7211.2	4.1			

Df: degrees of freedom. SS: sums of squares. MS: Mean squares. F: F ratio. P: p value. % Exp: percentage of variance explained. Trans: transgenic line. Accession/Trans: factor "transgenic line" nested within factor "accession". We tested the contributions of the accessions and of the insert position (transgenic line) to variation of *GI* peak time and period length with an ANOVA using accession and transgenic line nested within accession as factors. The statistical tests were performed with the data from the 39 accessions for which at least two transgenic lines had been scored (see *Methods*).

Table S3. Analysis of the technical variability observed for *GI* peak time.

	Col-0 X Lip-0 F2 population		Accessions							
			experiment 1		experiment 2		experiment 3		experiment 4	
	plate 1	***plate 2***	Col-0	Lip-0	Col-0	Lip-0	Col-0	Lip-0	Col-0	Lip-0
Mean	***11.06***	***11.69***	10.55	11.29	10.72	11.68	10.81	12.01	10.97	12.72
s.d.	***0.77***	***0.98***	0.46	0.43	0.68	0.54	0.51	0.93	0.55	0.48
n	***48***	***48***	48	47	36	36	32	32	32	32

The variation of *GI*::*LUC* peak time of expression was analyzed in the Col-0 X Lip-0 *GI*::*LUC* F2 population, and compared with the variation observed in Col-0 *GI*::*LUC* and Lip-0 *GI*::*LUC* populations of a similar size. Plate 1 and plate 2 refer to independent experiments in which the Col-0 X Lip-0 *GI*::*LUC* F2 population was tested. Four biological replicates (experiments 1 to 4) of Col-0 *GI*::*LUC* and Lip-0 *GI*::*LUC* populations were analyzed. Data from LDs of 16 h were used for these comparisons as maximum variation between accessions and within the F2 population were detected in this condition. This analysis shows that there was more phenotypic variability within the F2 population than within populations of Col-0 *GI*::*LUC* and Lip-0 *GI*::*LUC*. Therefore, technical variability alone did not account for the variation observed in the F2. Further statistical treatments of these data are presented in Table S4. s.d.: standard deviation. *n*: number of individuals per experiment.

Table S4. Statistical analysis of *GI::LUC* peak time of expression in Col-0 and Lip-0.

	Df	F	P	% Exp
Genotype	1	250.685	<0.001	39.64899
Experiment	3	30.525	<0.001	14.48395
Residuals	290			45.86706

Df: degrees of freedom. F: F ratio. P: p value. % Exp: percentage of variance explained. Data were obtained from four biological replicates in which Col-0 *GI*::*LUC* and Lip-0 *GI*::*LUC* were grown in LDs of 16 h, as previously described in Table S3. The F ratios and p values were determined with a two way ANOVA using genotype and experiment as factors.

Table S5. QTL detection summary in the F2 population.

	Chromosome	Position cM	LOD score	% Expl	Additive effect	QTL name
GI peak time LD1	2	40.12	2.59	7.4	-0.42	*TOG1*
	3	1.58	3.54	10.2	0.46	*TOG2*
	4	0	0.53	1.4	0.17	*TOG3*
	5	60.23	1.22	3.4	-0.23	*TOG4*
GI peak time LD2	2	40.12	2.52	7.1	-0.30	*TOG1*
	3	1.58	1.78	4.9	0.36	*TOG2*
	4	0.00	2.32	6.6	0.17	*TOG3*
	5	60.23	0.62	1.6	-0.26	*TOG4*

cM: centimorgan. LOD: Likelihood of odds. % Expl: percentage of variance explained. For the additive effect, negative values indicate that the Lip-0 allele delays *GI* peak time. LD1 and LD2: first and second consecutive LD cycles of 16 h light / 8 h dark. Note that the effects of *TOG3* and *TOG4* were not significant in the F2 population but a LOD peak was detected at *TOG3* in LD2, and a weak but consistent effect was detected at *TOG4* in both LDs. Based on these results the effects of *TOG3* and *TOG4* were further tested in F3 families and these experiments confirmed *TOG3* and *TOG4* (Fig. S4). All the QTLs were finally confirmed in NILs (Fig. S5).

Table S6. Size of the *TOG* introgressions in the NILs presented in Fig. S5.

			Introgression				
interval	polymorphism		*TOG1*	*TOG2*	*GI::LUC* transgene	*TOG3*	*TOG4*
large interval	upper	position	6402846	-	2236791	-	18888298
		reference	CIW3	-	ossowski_455674	-	PERL1058386
	lower	position	10032183	4141096	4141096	269026	-
		reference	PERL0356500	PERL0446897	PERL0446897	FRI	-
	interval size	size in bp	3629337	4141096	1904305	269026	9821054
small interval	upper	position	7203681	-	3679535	-	17154448
		reference	PERL0336650	-	MASC01999	-	PERL1026858
	lower	position	9529916	3679535	4141096	195281	-
		reference	PERL0353940	MASC01999	PERL0446897	PERL0659066	-
	interval size	size in bp	2326235	3679535	461561	195281	8087204

The positions and reference numbers of the polymorphisms that define the limits of the introgressions are indicated. Upper and lower polymorphisms define the upper and lower limits of the intervals. The large interval is defined by the two upper and lower polymorphisms that fall outside the introgression. The small interval is defined by the two upper and lower polymorphisms that fall inside the introgression. The upper limits of *TOG2* and *TOG3* are the top of chromosome III and IV, respectively, and the lower limit of *TOG5* is the bottom of chromosome V, which is why there is no polymorphism information at these positions. The *GI::LUC* transgene came from the Lip-0 accession in the original cross and was linked to *TOG2* (Fig. S3). Therefore, the table also provides the size of the introgression when the transgene was isolated from *TOG2* Lip-0. Marker CIW3 is available on the TAIR website (www.arabidopsis.org). The FRI marker in Col-0 was described in (4). Reference numbers of the SNPs were found on the TAIR website.

Table S7. *GI* peak time, *GI* expression level at peak time (*GI* max) and period length in DD measured in the NILs.

***GI* peak time (h)**	SD8	SD10	LD14	LD16
NIL1	8.758 ± 0.0478	10.654 ± 0.0493	12.863 ± 0.101	13.095 ± 0.103
NIL2	8.736 ± 0.0478	10.68 ± 0.0489	12.45 ± 0.101	12.882 ± 0.103
NIL3	8.703 ± 0.0493	10.714 ± 0.0489	12.835 ± 0.102	13.348 ± 0.103
NIL4	8.698 ± 0.0478	10.698 ± 0.0493	12.551 ± 0.103	13.023 ± 0.103
NIL5	8.852 ± 0.0478	10.667 ± 0.0489	12.351 ± 0.101	12.155 ± 0.103
NIL6	8.857 ± 0.0482	10.68 ± 0.0493	12.362 ± 0.114	12.543 ± 0.103
NIL7	8.887 ± 0.0478	10.762 ± 0.0489	12.829 ± 0.101	12.899 ± 0.103
NIL8	8.808 ± 0.0478	10.813 ± 0.0489	12.935 ± 0.101	12.88 ± 0.103
NIL9	8.814 ± 0.0486	10.682 ± 0.0489	12.774 ± 0.101	13.22 ± 0.104
NIL10	8.883 ± 0.0478	10.829 ± 0.0489	12.81 ± 0.103	13.128 ± 0.105
NIL11	8.901 ± 0.0478	10.637 ± 0.0557	12.881 ± 0.101	13.381 ± 0.103
NIL12	8.843 ± 0.0482	10.626 ± 0.0489	12.447 ± 0.101	12.885 ± 0.103
n / genotype	**60**	**60**	**60**	**60**
p	**0.012**	**0.06**	**<0.001**	**<0.001**

***GI* max (cps)**	SD8	SD10	LD14	LD16
NIL1	2487.267 ± 328.56	5324.133 ± 445.704	4220 ± 369.05	5931.507 ± 432.358
NIL2	3270.133 ± 328.56	3789.133 ± 445.704	4865.533 ± 369.05	6210.933 ± 423.962
NIL3	3773 ± 328.56	5164.533 ± 445.704	5435.333 ± 369.05	6982.387 ± 432.358
NIL4	3236.4 ± 328.56	4545.667 ± 445.704	6126.224 ± 378.713	7382.8 ± 432.358
NIL5	4729.8 ± 328.56	6943.805 ± 453.411	6340.158 ± 375.434	8507.897 ± 427.799
NIL6	3748.267 ± 328.56	4270.8 ± 445.704	6354.792 ± 416.355	7772 ± 423.962
NIL7	2677.6 ± 328.56	4469.467 ± 445.704	5020.641 ± 375.434	5859.194 ± 427.799
NIL8	2536.2 ± 328.56	4449.667 ± 445.704	5086.133 ± 369.05	6742.2 ± 423.962
NIL9	2653.267 ± 328.56	4596.8 ± 445.704	4371.733 ± 369.05	5371.6 ± 423.962
NIL10	3155.2 ± 328.56	3797.267 ± 445.704	5206.733 ± 369.05	6337.267 ± 423.962
NIL11	2688.733 ± 328.56	3423.588 ± 502.825	4742.933 ± 369.05	5801.4 ± 423.962
NIL12	2483.333 ± 328.56	3584.533 ± 445.704	4054.733 ± 369.05	5473.236 ± 427.799
n / genotype	**60**	**60**	**60**	**60**
p	**<0.001**	**<0.001**	**<0.001**	**<0.001**

period length (h)	SD8	SD10	LD14	LD16
NIL1	26.396 ± 0.264	26.151 ± 0.303	25.958 ± 0.372	25.754 ± 0.422
NIL2	26.236 ± 0.259	26.038 ± 0.303	25.801 ± 0.345	25.119 ± 0.314
NIL3	26.027 ± 0.253	26.083 ± 0.309	25.373 ± 0.364	25.103 ± 0.314
NIL4	25.703 ± 0.261	25.305 ± 0.298	25.083 ± 0.368	24.946 ± 0.32
NIL5	26.215 ± 0.256	26.071 ± 0.298	25.637 ± 0.354	25.405 ± 0.297
NIL6	26.417 ± 0.263	26.03 ± 0.303	25.948 ± 0.391	25.994 ± 0.308
NIL7	25.862 ± 0.251	25.989 ± 0.309	26.001 ± 0.357	25.554 ± 0.305
NIL8	26.603 ± 0.28	26.231 ± 0.309	26.102 ± 0.361	26.45 ± 0.317
NIL9	26.452 ± 0.253	26.534 ± 0.318	26.945 ± 0.351	26.023 ± 0.309
NIL10	26.516 ± 0.253	26.4 ± 0.306	25.836 ± 0.345	25.654 ± 0.33
NIL11	26.73 ± 0.268	26.212 ± 0.361	26.298 ± 0.345	25.753 ± 0.297
NIL12	26.57 ± 0.258	27.099 ± 0.309	26.131 ± 0.348	25.768 ± 0.309
n / genotype	**60**	**60**	**60**	**60**
p	**0.148**	**0.043**	**0.07**	**0.037**

Least Square means ± s.e.m. are shown. *n* denotes number of individuals assayed per NIL per photoperiod. Data were obtained from 5 independent biological replicates per condition. 12 individuals were assayed per genotype per experiment (2880 plants in total). The contribution of genotypic variation to variation of the phenotype was determined with two way ANOVA with genotype and experiment as factors. p indicates statistical significance of the F ratio.

Table S8. Primers used for qRT PCR.

gene	forward primer	reverse primer
GI	TGGTTTCCTCTTGGATTCAT	CTGTTCAGACGTTCAAAGGC
PIF4	CGGAGTTCAACCTCAGCAGT	ACCGGGATTGTTCTGAATTG
LUC	AAGCGGTTGCCAAGAGGTTCC	CGCGCCCGGTTTATCATC
TUB2	ACACCAGACATAGTAGCAGAAATCAAG	ACTCGTTGGGAGGAGGAACT
IPP2	GTATGAGTTGCTTCTGGAGCAAAG	GAGGATGGCTGCAACAAGTGT